Revue suisse de Zoologie 121 (3): 425-439; septembre 2014

Current status of the Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam with implications for conservation measures

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Current status of the Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam with implications for conservation measures. - The Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 is a monotypic species, with a distribution range restricted to small and isolated areas in southern China and northern Vietnam. Habitat destruction and illegal poaching are the main causes of alarming population declines and even extinction of some wild populations in China. While the Chinese population was estimated to comprise only 950 individuals in 2004, the existing status of the Vietnamese population remains unknown, since its discovery in 2002. Our work provides the first estimation of the population size of *S. crocodilurus* in Vietnam, which is essential baseline data for future conservation strategies. Our field research revealed a dramatically small population size of less than 100 mature individuals. This value falls substantially below published threshold sizes of several thousand individuals, required for the long-term persistence of a species. Our research highlights the urgent need to improve the conservation activities for this species in its natural habitats and suggests means for a translocation program to restore (minimum viable sizes of) the wild populations in northern Vietnam.

Keywords: Population size - PIT tags - MVP - Conservation planning - Restoration - Southeast Asia - Yen Tu Mountain.

INTRODUCTION

The Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 (Fig. 1) is the only living representative of the monotypic family Shinisauridae and recognized as a true "living fossil" (Hu *et al.*, 1984; Huang *et al.*, 2008; Le & Ziegler, 2003; Zhao *et al.*, 1999). The species has specific habitat requirements such as undisturbed rocky streams within the evergreen rainforests with a known geographic range restricted to few small and isolated areas in northern Vietnam and southern China (Huang *et al.*, 2008; Le &

Manuscript accepted 24.06.2014

FIG. 1
Shinisaurus crocodilurus in its natural habitat in Tay Yen Tu NR, Vietnam. Photo: M. van Schingen.

Ziegler, 2003; van Schingen *et al.*, 2014). The Crocodile Lizard is threatened by extinction, with illegal poaching and habitat loss being recognized as the major threats to this species in China. Its resemblance to a crocodile makes it a desired target species on the international pet market and its reduced activity and low metabolism makes the species an easy prey of illegal poachers (Huang *et al.*, 2008; Le & Ziegler, 2003; Wang *et al.*, 2009). As a result *S. crocodilurus* is experiencing alarming population declines and even extirpation at some localities in China (Huang *et al.*, 2008). In 1990 the species was finally listed by the Committee on the International Trade in Endangered Species (CITES) on appendix II in an attempt to diminish the trade with the species and to minimize further population declines (Huang *et al.*, 2008). However, a study conducted in 2004 on the Chinese population concluded that only 950 individuals remained in the wild and revealed dramatic local declines of up to 90% in 25 years (Huang *et al.*, 2008). Today the populations of *S. crocodilurus* in China have likely declined even more, while the existing status of the Vietnamese subpopulation remains unknown since its discovery in 2002 (Le & Ziegler, 2003).

In Vietnam the species was reported from three different localities, all in areas with some degree of protection: Tay Yen Tu Nature Reserve (NR) in Bac Giang Province, and Yen Tu NR and Dong Son - Ky Thuong NR in Quang Ninh Province (Le & Ziegler, 2003; Hecht *et al.*, 2014; van Schingen *et al.*, 2014), being at least 10 km

apart from each other. All three sites are part of the last remaining contiguous lowland rainforest of Northeast Vietnam, which harbours a unique fauna not being found elsewhere in the country and which is zoogeographically related to southern China (Nguyen, 2011).

Appropriate estimations of the population size provide essential information for the classification of the threat level of a species and are crucial for wildlife management and management of the long-term survival of populations and species (Reed *et al.*, 2003; Traill *et al.*, 2007). Several studies support the notion that the size of the "minimum viable population" (MVP) is in reality much higher than the threshold sizes proposed by the IUCN and lie in the dimension of several thousand individuals (e.g., Reed *et al.*, 2007; Traill *et al.*, 2010).

This study includes a preliminary evaluation of the existing status of *S. crocodilurus* in Vietnam and provides information and evidence for the necessity of immediate conservation measures to protect this species in its natural habitat. Subsequently we provide recommendations for future conservation strategies of *S. crocodilurus*.

METHODS

Study sites: The surveyed sites were selected based on the previous discovery of three subpopulations of *S. crocodilurus* in Yen Tu NR, Uong Bi District, Quang Ninh Province (21°06' - 21°11'N, 106°37' - 106°43'E) in 2002; in Tay Yen Tu NR, Son Dong District, Bac Giang Province (21°09' - 21°23'N, 106°38' - 107°02'E) in 2010 and the recent discovery in Dong Son - Ky Thuong NR in Hoanh Bo District, Quang Ninh Province (21°05' - 21°12'N, 106°56' - 107°13'E) in 2013 (Le & Ziegler, 2003; Hecht *et al.*, 2014; van Schingen *et al.*, 2014), see Fig. 2. Tay Yen Tu and Yen Tu NRs are contiguous forest areas with Mount Yen Tu forming the highest peak (1068 m a.s.l.) and are linked in the East to the Dong Son - Ky Thuong NR by a forest corridor. The vegetation is dominated by evergreen broadleaf forest and intermixed with bamboo forest within the Dong Son - Ky Thuong NR. The study sites are part of the last remaining evergreen forest in Northeast Vietnam, which has been substantially cleared off from the eastern side of the Red River.

Field survey: Field surveys were conducted in June and July 2013, during the non-hibernation season of the Crocodile Lizard. Due to its strong association with lentic habitats and a diurnal life-mode, the riverine vegetation of selected rocky streams was sampled upstream during repeated night excursions between 6:45 and 10:30 pm, when animals were expected to rest on perches above the water. Captured animals were tagged and released on the exact same place on the following day between 12:00 am and 7:00 pm.

A total of 14 different stream transects were sampled, ranging from 515 to 3500 m in length. In the western side of the Yen Tu mountain range located within the Tay Yen Tu NR, six streams between elevations of 350-500 m a.s.l. were surveyed. On the eastern side of the Yen Tu range, four stream transects within the Yen Tu NR at elevations between 700-850 m a.s.l. and four streams in Dong Son - Ky Thuong NR at elevation between 200-350 m a.s.l. were surveyed. Coordinates and elevations of each captured individual were recorded with a GPS.

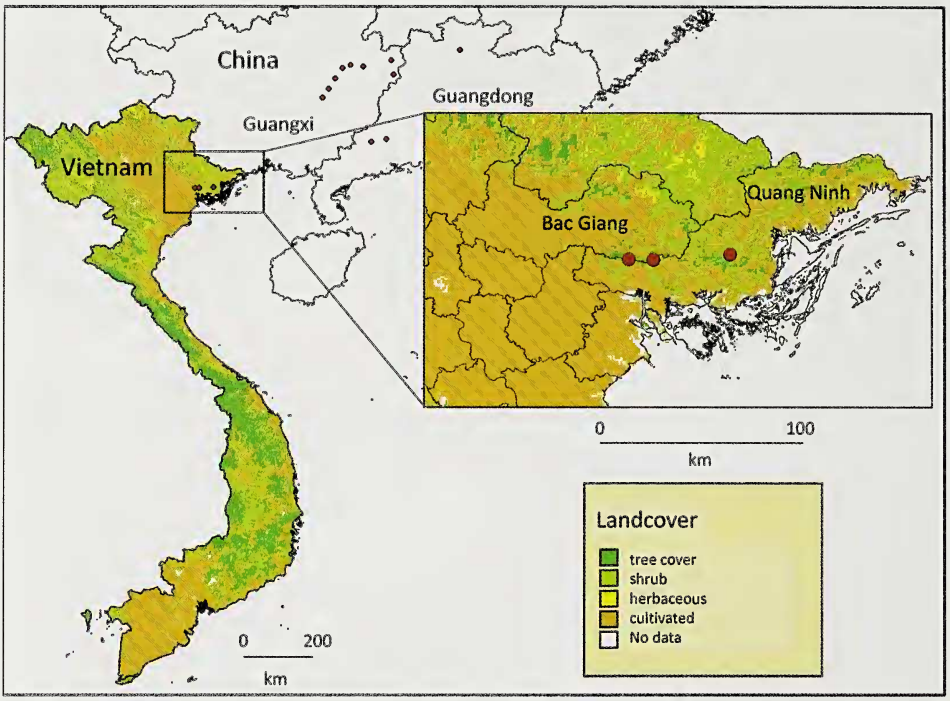

FIG. 2
Map of the current distribution of *Shinisaurus crocodilurus*. Red dots represent occurrence records.

Based on snout vent-length (SVL), individuals were classified into three different age groups, viz. juvenile (SVL < 100 mm), subadult (100 mm ≤ SVL < 140 mm) and adult (SVL ≥ 140 mm). Injuries were recorded, with special attention to the caudal region that was used as a measure of multivariate stressors.

Tagging: For the long-term monitoring of population dynamics of the species, individuals were tagged with passive integrated transponder (PIT) tags. PIT tags are commonly applied both in studies of vertebrates and invertebrates (Smyth & Nebel, 2013). Its use has established as a safe and reliable method, with low mortality rates and virtually no implications on moving speed, growth rate and health of the animal (Keck, 1994; Smyth & Nebel, 2013). It is also recommended by CITES to identify captive-bred animals, and to monitor illegal harvests as well as the international trade of species at risk (Gibbons & Andrews, 2004).

A unique PIT tag (ISO FDX-B, 9 x 1.4 mm) was inserted under the skin on the left body side behind the shoulder of each captured individual. The puncture was closed with petrolatum. The functioning of all microchips had been tested earlier with a reader (Breeder Reader LC, Planet ID GmbH). Tagged individuals did not show any signs of injury resulting from the injection and were released within 24 hours of capture. Recaptures were identified and released immediately.

Calculation: The population size was estimated by applying a capture-recapture method modified for *S. crocodilurus* by Huang *et al.* (2008). Accordingly, we calcu-

lated an "invisibility rate" which was adopted for *S. crocodilurus* to compensate for animals present but not seen during the surveys. This method was selected to obtain comparable data to the estimates on the Chinese population. The calculation of the "invisibility rate" was based on three consecutive time surveys in intervals of 1-12 days within the Tay Yen Tu NR: $N = \sum [n (1 + i)]$, where N is the total population size, n is the number of observed individuals along a stream transect and i is the "invisible rate" index: $i = [\sum(b_n - a_n)] / \sum a_n$, where a_n is the number of observed individuals in the transect n during the first survey and b_n is the total number of observed individuals in transect n. The transect n equals the surveyed stream.

Statistical analyses: Statistical analysis was performed with the program PAST (Hammer *et al.*, 2001). A Chi^2-Test was applied to test for differences among age classes and the occurrence of injuries between different localities. Significant difference was declared for $p < 0.05$ ($p < 0.05$ = *, $p < 0.01$ = ** and $p < 0.001$ = ***).

RESULTS

Population size: During the field research *S. crocodilurus* was found in seven different streams of three nature reserves. A total of 62 individuals were captured and 32 recapture events took place during the survey. Based on a calculated invisibility rate index of 1.35 the total population in Vietnam was estimated to comprise about 98 individuals, from which only 59 were considered to be mature (Tab. 1). The highest density of *S. crocodilurus* was found in Tay Yen Tu NR (28 individuals per km transect stream), while densities were lowest in Dong Son - Ky Thuong NR, ranging from 1 to 6 individuals per km transect stream (Tab. 2).

Population structure: The number of individuals capable of reproduction is crucial for the survival of the population and thus serves as measure to evaluate the endangerment of species (IUCN, 2013). *S. crocodilurus* reaches maturity at about three years (Yu *et al.*, 2009). The age is highly related to the animals size, whereby the snout-vent length proved to be the most appropriate measure corresponding to body size in lizards, as tails are prone to be injured (Meiri, 2010). A frequency histogram of the snout-vent length of captured *S. crocodilurus* revealed two maxima at 85 and 150 mm (Fig. 3A). This pattern shows that the wild population investigated in our study consisted of relatively high numbers of juveniles and young adults, but only of few subadults and big adults. A conspicuous reproduction success was observed in the Tay Yen Tu NR, related to the high proportions of juveniles, which represented 57.5% of this subpopulation (Fig. 4A). A significantly smaller success was reported in Yen Tu (8.3%) and in Dong Son - Ky Thuong (9.1%) NRs ($Chi^2 = 19.31$, $df = 4$, $p = 0.0007$; Fig. 4A). Taking into account the whole Vietnamese population, the number of adults and juveniles was represented with high percentages of about 47.6% and 39.7%, respectively, while subadults only contribute with about 12.7% (Fig. 4A).

Besides spatial differences in the population structure, high temporal fluctuations were also observed. In comparison to a previous field survey conducted by our team in June and July 2010 in Tay Yen Tu NR, the recent survey revealed: a more than tenfold increase in the number of juveniles; while the frequency of observed adults had almost doubled ($Chi^2 = 8.591$, $df = 2$, $p = 0.0136$; Fig. 4B).

TAB. 1. Estimated wild population size of *Shinisaurus crocodilurus* in Vietnam.

Nature Reserve	Tay Yen Tu	Yen Tu	Dong Son - Ky Thuong
$Subtotal_{mature}$ (all)	20 (51)	17 (21)	22 (26)
$Total_{mature}$ (all)		59 (98)	

TAB. 2. Abundances of observed *Shinisaurus crocodilurus* in Vietnam: *Ad* = adults, *Sub* = subadults, *Juv* = juveniles.

Nature Reserve	Transect [m]	Ad_{obs}	Sub_{obs}	Juv_{obs}	$Total_{obs}$	$Density_{obs}$ [Ind_{obs}/ km]
Tay Yen Tu						
1	842	3	2	2	7	8.3
2	1200	11	1	21	33	27.5
Yen Tu						
1	514	8	0	0	8	15.6
2	1600	2	0	1	3	1.9
Dong Son - Ky Thuong						
1	650	2	1	1	4	6.2
2	3500	1	3	0	4	1.1
3	830	2	0	0	2	2.4

A comparison of tail conditions among the three nature reserves showed that 25, 58 and 70 % of the observed individuals from Tay Yen Tu NR, Yen Tu NR and Dong Son - Ky Thuong NR, had regenerated tails, respectively ($Chi^2 = 8.036$, $df = 2$, $p = 0.018$; Fig. 3B). The habitats within Dong Son - Ky Thuong NR were the closest to local villages, had the lowest elevations (200-350 m a.s.l.) and thus were more easy to access, in comparison to the other locations. Furthermore in this area the streams were broader, less vegetated and did not comprise as many waterfalls, backwater pools and shelters like in the other two reserves. The highest visual encounters of sympatric occuring reptiles such as the Waterdragon *Physignathus cocincinus* were also found within the habitat of *S. crocodilurus* in Dong Son - Ky Thuong NR.

CONSERVATION STATUS

Threats: The major threats to the population of *S. crocodilurus* in Vietnam are habitat loss and habitat alterations caused by intensive coal mining and illegal timber logging (Ziegler *et al.*, 2008; pers. obs.). Coal-mining leads not only to fragmentation but also results in the contamination of the forest floor and forest streams, threatening the water-associated organisms (Fig. 6A). In Tay Yen Tu NR, the species' habitat was seriously disturbed. The forest has been opened throughout the nature reserve in order to build roads and facilitate coal-mining (Fig. 6B). The mining area has been steadily expanding and meanwhile almost touched a stream habitat of *S. crocodilurus*. During one night survey in 2013, a huge hydraulic excavator was observed working in a distance of less than 50 m to a habitat stream of *S. crocodilurus*. In addition, huge parts of the forest have been cleared by slash and burn agriculture or have been harvested for the paper industry (Fig. 5). Habitat destruction was also hazardous in the Dong Son - Ky Thuong NR, caused in main parts by the activities of Hoanh Bo Forest Enterprise

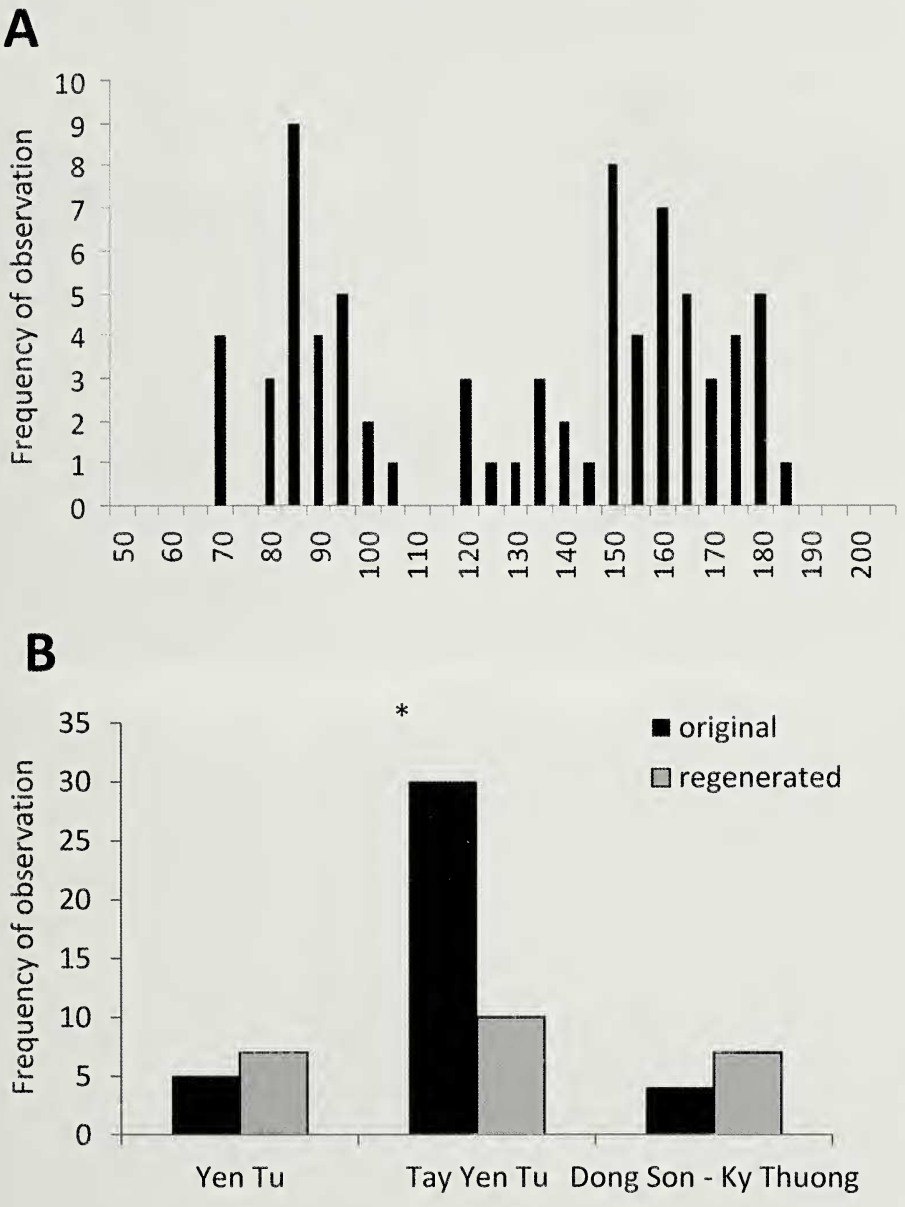

FIG. 3

Population structure of *Shinisaurus crocodilurus* in Vietnam. (A) Frequency histogram of snout-vent length of all encountered animals; (B) Frequency of individuals with original or regenerated tails for each nature reserve, $p < 0.05$.

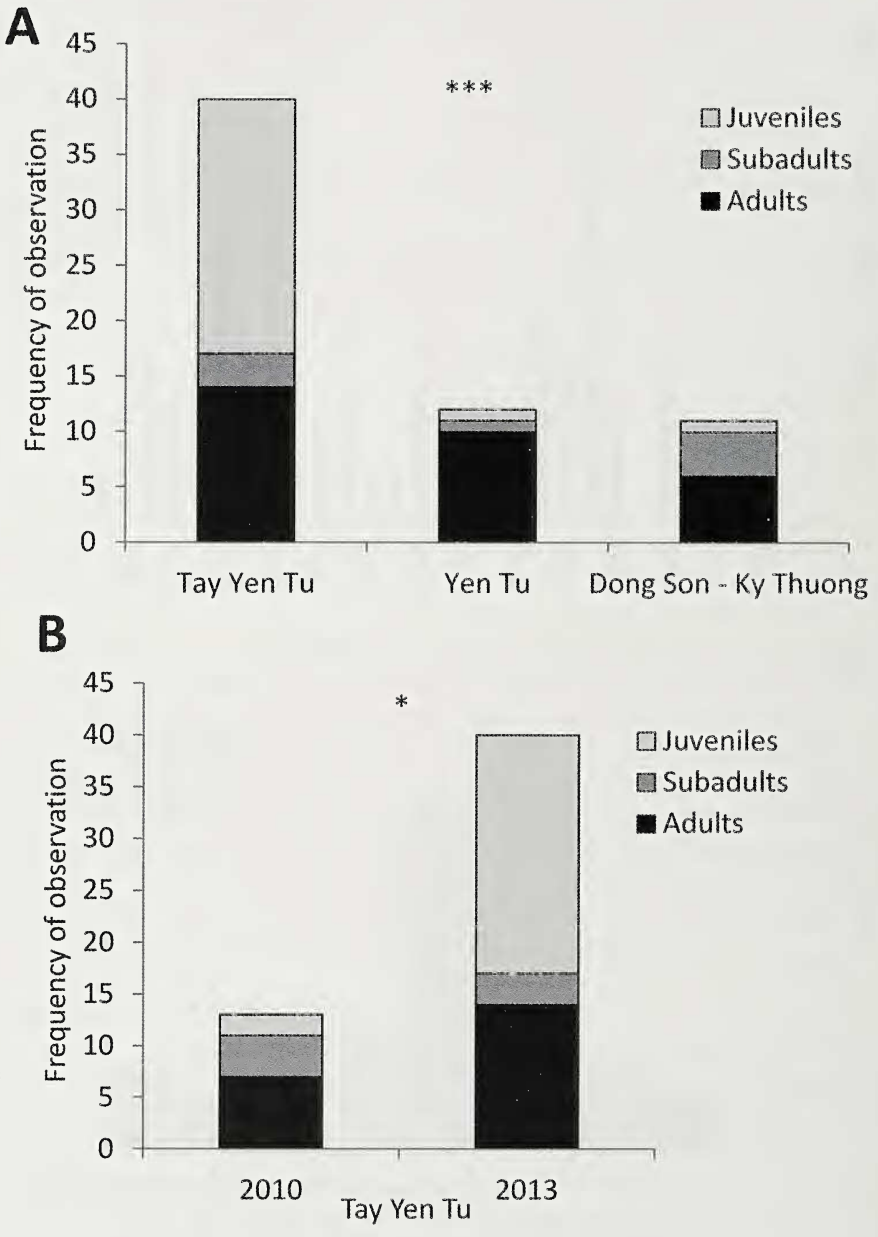

FIG. 4

Distribution of different age classes of *S. crocodilurus* in Vietnam. (A) Frequency of observed juveniles, subadults and adults from three nature reserves, $p < 0.001$; (B) Frequency shift (of juveniles, subadults and adults) in Tay Yen Tu NR between 2010 and 2013, $p < 0.05$.

FIG. 5

Slash and burn practices in Tay Yen Tu NR, Vietnam. (A) example of forest fire; (B) burned area cleared for agricultural purposes or exploration by the paper industry. Photos: M. Bernardes & M. van Schingen.

(see also Birdlife International, 2004). The construction of logging roads throughout the Dong Son - Ky Thuong NR has facilitated illegal logging and increases the accessibility of almost all areas within the nature reserve (Tordoff *et al.*, 2000). We could prove the observation of Tordoff *et al.* (2000) that hunting posed a severe threat to the biodiversity in Dong Son - Ky Thuong, as our interviews with local people showed that they indeed randomly collect amphibians and reptiles within the nature reserve as food source, and that Crocodile Lizards were collected for traditional medicine (Fig. 6C). Le & Ziegler (2003) already reported that illegal poaching for the pet trade threatens the Vietnamese population. Sold as "baby crocodile" for 100.000 to 200.000 Vietnam Dong (about 7-15 US Dollars), the Crocodile Lizard is a big seller especially among tourists. This observation agreed with our findings that Vietnamese specimens apparently also ended up in the international pet trade, as they are being offered already for sale in the internet (e.g., Doelle *in lit.*, 2013; *pers. obs.*). The high demand for the species, especially in European countries immensely increases the hunting pressure on the wild populations.

DISCUSSION

The persistence of populations in the wild depends on the size of viable subpopulations and the exchange and speed of recolonization from nearby habitats, with particularly small and range-restricted populations being highly prone to extinction in various animal species (Hanski, 1991; Reed *et al.*, 2003; Traill *et al.*, 2007). In terms of the ongoing alarming global loss of biodiversity, guidelines to link extinction risk to population size have high priority in conservation biology (Lawton & May, 1995; Reed *et al.*, 2003; Shaffer *et al.*, 2002). The concept of a 'minimum viable population' (MVP; Shaffer, 1981) has been frequently applied in terms of species recovery and conservation management programs, with relevance to the IUCN Red List's criteria concerning small and range-restricted populations (e.g., Clark *et al.*, 2002; Reed *et al.*, 2003; Traill *et al.*, 2007, 2010). The MVP is defined as the smallest threshold size, which is required for a population or a species to have a predetermined probability of persistence for a given length of time (Reed *et al.*, 2003; Shaffer, 1981). Experiments on isolated subpopulations revealed a local extinction of subpopulations with $n < 50$ and persistence with $n > 50$ individuals (Berger, 1990). With respect to reptiles and amphibians Traill *et al.* (2007) summarized MVPs ranging from 3611 to 6779 individuals and stated that MVPs generally lie in the range of several thousand individuals. Reed *et al.* (2003) concluded that a population size of at least 7000 adults in any vertebrate is required to cope with evolutionary and demographic constraints in the long-term. The population size of *S. crocodilurus* in Vietnam was preliminary estimated to comprise about 59 mature individuals and thus being dramatically smaller than the Chinese population with 950 estimated individuals in 2004 (Huang *et al.*, 2008). The high incidence of juveniles, most concise within the Tay Yen Tu NR implicates that the reproduction capability of the population is not entirely constrained by certain stressors, but rather secondary hazards as habitat degradation and poaching are assumed to limit the population persistence in the long-term, comparable to the Chinese population (Huang *et al.*, 2008).

FIG. 6

Main threats to *S. crocodilurus* in Vietnam. (A) Coal-mining exploration close to the species' habitat; (B) Opening of the forest with roads to facilitate coal-mining throughout the nature reserves; (C) Preserved *S. crocodilurus* in alcohol, used for traditional medicine in Quang Ninh Province. Photos: M. Bernardes & M. van Schingen.

The order of injuries in specimens differed among the three sites and was highest within the Dong Son - Ky Thuong NR. An unfavourable habitat structure, the occurrence of predators or competitors and human impacts might be potential reasons for higher rates of violated specimens in this reserve.

Furthermore, our study revealed that *S. crocodilurus* is strongly sedentary, as no migration between habitat streams in striking distance was proved within three years. In long-term view the restricted migration ability might reduce the gene flow and thus endanger the continuance of the species. The extremely small subpopulation sizes of about 20 mature individuals within each nature reserve make the species prone to fall into an extinction vortex (Gilpin & Soulé, 1986). Strong fluctuations within populations make them especially prone to extinction, even though populations generally underlie some level of fluctuations (e.g., Bjørnstad & Grenfell, 2002; Ranta *et al.*, 2006). In this context our study revealed that the subpopulation from Tay Yen Tu NR had more than doubled from 2010-2013, including a more than 11-fold increase in the proportion of juveniles. This high incidence of juveniles was observed nowhere else in Vietnam. However, the duration of survival appeared strongly restricted as only one of 13 individuals, marked in 2010 was recaptured in 2013. Since *S. crocodilurus* reaches sexual maturity only after three years, the survival during this period is crucial for the maintenance of its populations (Zhang, 2006; Yu *et al.*, 2009).

CONSERVATION MEASURES

Habitat protection: Based on the observation of various threats to the habitat of *S. crocodilurus* in Vietnam (e.g., continuously expanding coal-mining area in the direction of the habitat streams, habitat fragmentation from roads made for coal exploration and logging companies, forest clearance and natural forest fires), we strongly recommend a protection status elevation of the nature reserves in close collaboration with the authorities of the reserves. As many Crocodile Lizard populations are distributed outside or within the buffer regions of the NRs (van Schingen *et al.*, 2014), an extension of the protected area network should be further considered. Apart from the protection of the macrohabitat, we recommend that at least the habitat streams need higher protection to enable the long-term persistence of the species. An agreement with the operators of local coal-mining companies is necessary to protect the minimum area required for the survival of the population, which would be feasible as the species is strongly sedentary and restricted to few specific streams (Ning *et al.*, 2006; van Schingen *et al.*, 2014). Roads, which are increasingly created throughout Tay Yen Tu and Dong Son - Ky Thuong NRs to facilitate coal-mining and timber logging (Tordoff *et al.*, 2000; *pers. obs.*), should be directed around the habitat streams.

Wildlife trade control: To control the trade, an enhancement of the conservation status of *S. crocodilurus* by the assessment of the species for the IUCN Red List is recommended just as an upgrade of the CITES appendix. We also propose to include *S. crocodilurus* in the list of protected species in Vietnam. Illegal collections for the pet trade should be controlled by forest ranger stations through patrols at touristic sites like Tay Yen Tu and Yen Tu NRs. As *S. crocodilurus* is a habitat specialist (Ning *et al.*, 2006; van Schingen *et al.*, 2014; Wu *et al.*, 2007), only occurring along specific streams, this measure would be feasible and effective. A public awareness campaign

(e.g., brochure, poster, signboard) should be conducted for local communities inside protected areas and within their buffer zones.

Population restoration: First molecular analysis of the extant subpopulations revealed no significant genetic difference (Ziegler *et al.*, 2008). However, a broader genetic analysis to evaluate the closer taxonomic relationships of the extant subpopulations is recommended, as discrepancies would have a strong impact on the risk of extinction of subpopulations or even different taxa and would require a drastic enhancement of the conservation status of *S. crocodilurus* in Vietnam. However, the estimated total population size (China: 950 + Vietnam: 59) already falls below reported threshold sizes in the magnitude of several thousand individuals, which is required for the persistence of a species over a longer period (Traill *et al.*, 2010). Based on our findings a translocation program of the species to restore the wild population, particularly in Vietnam, is urgently recommended. Translocation, defined as movement of living organisms from one area to another (IUCN), forms an important tool in wildlife conservation (Germano & Bishop, 2008). Repatriations of animals into their natural habitats were frequently combined with captive-breeding programs at zoological parks (Scott & Carpenter, 1987). A restoration program of a subpopulation of *S. crocodilurus* in China (Luokeng NR, Guangdong Province) was already initiated in 2004 (Zhang, 2006). In addition, Vietnamese specimens originating from Yen Tu NR were already successfully bred in captivity at the Me Linh Biodiversity Station in Vinh Phuc Province, which was established in cooperation of the Institute of Ecology and Biological Resources, Hanoi and the Cologne Zoo, Germany (e.g., Ziegler *et al.*, 2013). Those individuals would be suitable for restocking the wild population. A reintroduction should proceed after IUCN standards and based on studies on the species' specific requirements. In addition, all captive bred specimens should be marked with PIT tags in order to monitor the development of introduced specimens and long-term population dynamics after the release to evaluate the restoration success. Moreover, tagging of wild individuals during the present study already provides a base for future long-term investigations of *S. crocodilurus* in Vietnam.

ACKNOWLEDGEMENTS

We thank the directorates of the Tay Yen Tu, Yen Tu and Dong Son - Ky Thuong nature reserves, Forest Protection Departments of Bac Giang and Quang Ninh provinces for support of our field work and issuing relevant permits. We are grateful to C. X. Le and T. H. Tran (Hanoi) for support of our research. Field work in Vietnam was partially funded by the Cologne Zoo, the European Union of Aquarium Curators (EUAC), University of Cologne, World Association of Zoos and Aquariums (WAZA), Harry Wölfel, Michael Zollweg and many private donations. Research of T. Q. Nguyen in Germany was funded by the Alexander von Humboldt Foundation (VIE 1143441).

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NOTE ADDED IN PROOF

While the present paper was in press, the inclusion of the species into the IUCN Red List and the list of protected species of Vietnam took place.

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